

What is claimed is:

- ✓ 5 1. Purified NEDD8-activating protein beta subunit. ✓
2. The purified NEDD8-activating protein beta subunit according to claim 1 having the amino acid sequence shown in Figure 1.
- ✓ 10 3. An NAE1-beta expression element. ✓
4. The NAE1-beta expression element selected from isolated or recombinant nucleic acid sequences encoding NAE1-beta or nucleic acid sequences specifically homologous or specifically complementary thereto, vectors comprising any such  
15 nucleic acid sequences, and recombinant expression units which express NAE1-beta, antisense transcripts, or dominant negative mutants thereof. ✓
- ✓ 5. A method for identifying NAE1BBMs comprising contacting purified NAE1-beta according to the invention and populations of molecules or mixed populations  
20 of molecules and determining the presence of molecules which bind specifically to NAE1-beta. ✓
- ✓ 6. An NAE1BBM identified by the method according to claim 5.
- ✓ 25 7. A method for determining the presence or absence and/or quantity of NAE1-beta, NAE1 heterodimer, or NAE1 heterodimer/NEDD8 complex in a biological sample, the method comprising providing a detectable NAE1BBM to a biological sample, allowing the detectable NAE1BBM to bind to NAE1-beta, NAE1 heterodimer, or NAE1 heterodimer/NEDD8 complex, if any is present in the

biological sample, and detecting the presence or absence and/or quantity of a complex of the detectable NAE1BBM and NAE1-beta, NAE1-heterodimer, or NAE1 heterodimer/NEDD8 complex.

✓ 5 8. A method for determining the presence or absence and/or quantity of NAE1-beta nucleic acid in a biological sample comprising providing to the biological sample a nucleic acid sequence which is specifically complementary to NAE1-beta nucleic acid.

✓ 10 9. A method for identifying modulating ligands of NAE1-beta comprising providing NAE1BBMs to an assay system for NAE1-beta participation in the NEDD8-activation/conjugation pathway, and determining whether such NAE1BBMs interfere with or enhance the ability of NAE1-beta to participate in the NEDD8-activation/conjugation pathway. ✓

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✓ 10. A modulating ligand of NAE1-beta. ✓

11. The modulating ligand of NAE1-beta identified by the method according to claim 9.

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12. The modulating ligand of NAE1-beta according to claim 10, which interacts with NAE1-beta to inhibit or enhance the formation of NAE1 heterodimer, the formation of NEDD8 adenylate, the formation of a thiol ester bond between NEDD8 and NAE1, and/or transfer of NEDD8 to NEDD8-conjugating enzyme.

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✓ 13. An antagonist of NAE1-beta which interferes with the expression of the NAE1-beta gene. ✓

✓ 14. A method for identifying NAE1ABMs comprising screening for NAE1ABMs

by contacting purified NAE1-alpha and populations of molecules or mixed populations of molecules and determining the presence of molecules which bind specifically to NAE1-alpha.

- ✓ 5 15. An NAE1ABM identified by the methods according to claim 14.
- ✓ 10 16. A method for determining the presence or absence and/or quantity of NAE1-alpha, NAE1 heterodimer, or NAE1 heterodimer/NEDD8 complex in a biological sample comprising providing a detectable NAE1ABM to a biological sample, allowing the detectable NAE1ABM to bind to NAE1-alpha, NAE1 heterodimer, or NAE1 heterodimer/NEDD8 complex, if any is present in the biological sample, and detecting the presence or absence and/or quantity of a complex of the detectable NAE1ABM and NAE1-alpha, NAE1-heterodimer, or NAE1 heterodimer/NEDD8 complex.
- ✓ 20 17. A method for detecting the presence or absence of NAE1-alpha nucleic acid in a biological sample in which NEDD8 conjugation is suspected, the method comprising providing to the biological sample a nucleic acid sequence which is specifically complementary to NAE1-alpha nucleic acid.
- ✓ 25 18. A method for identifying modulating ligands of NAE1-alpha comprising providing NAE1ABMs to an assay system for NAE1-alpha participation in the NEDD8-activation/conjugation pathway, and determining whether such NAE1ABMs interfere with or enhance the ability of NAE1-alpha to participate in the NEDD8-activation/conjugation pathway.
- ✓ 19. A modulating ligand of NAE1-alpha.

- ✓ 20. A modulating ligand which was identified by the method of claim 18.
21. The modulating ligand according to claim 19, which interacts with NAE1-alpha to inhibit the formation of NAE1 heterodimer, the formation of NEDD8  
5 adenylate, the formation of a thiol ester bond between NEDD8 and NAE1, and/or transfer of NEDD8 to NEDD8-conjugating enzyme.
- ✓ 22. An antagonist of NAE1-alpha which interferes with the expression of the NAE1-alpha gene.
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- ✓ 23. A method for modulating the activation and/or conjugation of NEDD8 comprising providing a modulating ligand of NAE1-beta or NAE1-alpha or a recombinant expression unit which expresses NAE1-beta or NAE1-alpha or an antagonist thereof to a biological system in which NEDD8 is conjugated to another  
15 protein.
- ✓ 24. A method for modulating auxin response in plants comprising providing a modulating ligand of NAE1-beta or NAE1-alpha or a recombinant expression unit which expresses NAE1-beta or NAE1-alpha or an antagonist thereof to a plant that is  
20 undergoing auxin treatment.
- ✓ 25. A method for modulating APP function and/or beta peptide accumulation in a biological system comprising providing a modulating ligand of NAE1-beta or NAE1-alpha or a recombinant expression unit which expresses NAE1-beta or NAE1-  
25 alpha or an antagonist thereof to a biological system.
- ✓ 26. A purified complex of NAE1-beta and NAE1-alpha, or a purified complex of portions thereof.

- ✓ 27. A purified complex of NAE1-beta, NAE1-alpha and NEDD8, or a purified complex of portions thereof.
- ✓ 28. An allelic variant of NAE1-alpha.
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- ✓ 29. An NAE1-alpha allelic variant expression element selected from isolated or recombinant nucleic acid sequences encoding NAE1-alpha, or nucleic acid sequences specifically homologous or specifically complementary thereto, vectors comprising any such nucleic acid sequences, and recombinant expression units which express
- 10 NAE1-beta or antisense transcripts or dominant negative mutants thereof.
- ✓ 30. Purified NEDD8-conjugating enzyme 1.
31. The purified NEDD8-conjugating enzyme 1 according to claim 30 having the
- 15 amino acid sequence shown in Figure 1.
- ✓ 32. An NCE1 expression element.
33. The NCE1 expression element according to claim 32 selected from isolated or
- 20 recombinant nucleic acid sequences encoding NCE1 or dominant negative mutants thereof, or expressing antisense transcripts thereof or nucleic acid sequences specifically homologous or specifically complementary thereto, vectors comprising any such NCE1 expression elements.
- 25 ✓ 34. A method for identifying NCE1BM comprising contacting purified NCE1 and populations of molecules or mixed populations of molecules and determining the presence of molecules which bind specifically to NCE1.
- ✓ 35. An NCE1BM identified by the method according to claim 34.

- ✓ 36. A method for determining the presence or absence and/or quantity of NCE1 or NCE1/NEDD8 complex in a biological sample, the method comprising providing a detectable NCE1BM to a biological sample, allowing the detectable NCE1BM to  
5 bind to NCE1, or NCE1/NEDD8 complex, if any is present in the biological sample, and detecting the presence or absence and/or quantity of a complex of the detectable NCE1BM and NCE1 or NCE1/NEDD8 complex.
- ✓ 37. A method for determining the presence or absence and/or quantity of NCE1  
10 nucleic acid in a biological sample comprising providing to the biological sample a nucleic acid sequence which is specifically complementary to NCE1 nucleic acid.
- ✓ 38. A method for identifying modulating ligands of NCE1 comprising providing NCE1BMs to an assay system for NCE1 participation in the NEDD8-  
15 activation/conjugation pathway, and determining whether such NCE1BMs interfere with or enhance the ability of NCE1 to participate in the NEDD8-activation/conjugation pathway.
- ✓ 39. A modulating ligand of NCE1. ✓
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- ✓ 40. The modulating ligand of NCE1 identified by the method according to claim 38.
41. The modulating ligand of NCE1 according to claim 39, which interacts with  
25 NCE1 to inhibit or enhance the formation of a thiol ester bond between NEDD8 and NCE1, and/or transfer of NEDD8 to NEDD8 target protein.
- ✓ 42. An antagonist of NCE1 which interferes with the expression of the NCE1  
gene. ✓

✓ 43. Purified NEDD8-conjugating enzyme 2. ✓

44. The purified NEDD8-conjugating enzyme 2 according to claim 43 having the  
5 amino acid sequence shown in Figure 4.

✓ 45. An NCE2 expression element. ✓

46. The NCE2 expression element according to claim 45 selected from isolated or  
10 recombinant nucleic acid sequences encoding NCE2 or dominant negative mutants thereof, or expressing antisense transcripts thereof or nucleic acid sequences specifically homologous or specifically complementary thereto, and vectors comprising any such NCE2 expression units.

15 47. A method for identifying NCE2BMs comprising contacting purified NCE2 and populations of molecules or mixed populations of molecules and determining the presence of molecules which bind specifically to NCE2. ✓

✓ 48. An NCE2BM identified by the method according to claim 47.

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✓ 49. A method for determining the presence or absence and/or quantity of NCE2 or NCE2/NEDD8 complex in a biological sample, the method comprising providing a detectable NCE2BM to a biological sample, allowing the detectable NCE2BM to bind to NCE2, or NCE2/NEDD8 complex, if any is present in the biological sample,  
25 and detecting the presence or absence and/or quantity of a complex of the detectable NCE2BM and NCE2 or NCE2/NEDD8 complex.

✓ 50. A method for determining the presence or absence and/or quantity of NCE2 nucleic acid in a biological sample comprising providing to the biological sample a

nucleic acid sequence which is specifically complementary to NCE2 nucleic acid.

- ✓ 51. A method for identifying modulating ligands of NCE2 comprising providing NCE2BMs to an assay system for NCE2 participation in the NEDD8-  
5 activation/conjugation pathway, and determining whether such NCE2BMs interfere with or enhance the ability of NCE2 to participate in the NEDD8-activation/conjugation pathway.
- ✓ 52. A modulating ligand of NCE2.
- 10 53. The modulating ligand of NCE2 identified by the method according to claim 51.
54. The modulating ligand of NCE2 according to claim 24, which interacts with  
15 NCE2 to inhibit or enhance the formation of a thiol ester bond between NEDD8 and NCE2, and/or transfer of NEDD8 to NEDD8 target protein.
- ✓ 55. An antagonist of NCE1 which interferes with the expression of the NCE1  
20 gene.
- ✓ 56. A purified complex of NCE1 and NEDD8, or a purified complex of portions  
thereof.
- ✓ 57. A dominant negative mutant of NCE1 or NCE2.
- 25 ✓ 58. An oligonucleotide that is specifically complementary to a portion of NCE1 or NCE2.